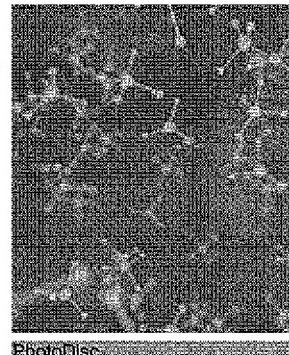


Exhibit 5



Detection of biological toxins is an extremely challenging technical problem of great societal importance. A very small concentration of a botulin toxin introduced into a city's water supply could be a serious health threat because of the toxin's potency.

Such difficult analytical problems can be solved by amplifying the signal, from a blip easily drowned out by all of the other constituents in a sample, to a strong peak that stands out clearly. Fourier transform instruments do this by acquiring signals repeatedly and adding them together. Geneticists use a tactic to amplify a gene fragment in preparation for sequencing: The polymerase chain reaction (PCR) exploits a natural enzyme, polymerase, that can recognize a specific fragment of DNA and then recruit cellular machinery to synthesize multiple copies of it.



PhotoDisc

In an April 16 advanced online publication to *Nature Biotechnology*, researchers at the Armed Forces Institute of Pathology, the Veterans Health Administration, and the US Food and Drug Administration describe a technique that uses a modified PCR—liposome PCR—to produce an ultrasensitive bioassay capable of detecting biotoxins at just a few molecules per sample.

The work uses PCR to amplify signals, with double-stranded DNA molecules locked within cell-like spheres called liposomes. Each liposome is formed from a bilayer of lipids that line up head to head to produce a thin shell with the hydrophilic (water-soluble) heads sandwiched between the hydrophobic (greasy, water insoluble) tails that line the exterior and interior surfaces of the bilayer. The researchers incorporated about 2,500 molecules of monosialoganglioside GM1 into the bilayer. These molecules bind cholera toxin beta subunit (CTBS).

To analyze for CTBS, the researchers first prepared a microtiter plate by attaching CTBS-specific antibodies to the surface of the wells to capture any toxin in a test solution. Next, they added the liposome reagent and incubated for 1 hour to allow the monosialoganglioside GM1 on the liposome surface to bind to any cholera toxin held fast by the antibodies on the well surface. Finally, they added a reagent to rupture the bound liposomes and release the double-stranded DNA. They took an aliquot from each well and performed PCR to confirm the presence or absence of DNA. If a cholera toxin was captured by an antibody lining the wall of a well and then targeted by a liposome, then the liposome's DNA—amplified by PCR—would be a clear indicator of the presence of the toxin.

The technique demonstrated exceptional sensitivity, with a detection threshold of 10 toxin molecules in a 150- μ L sample. In a more realistic test, the researchers spiked human urine and farm run-off water with CTBS and noted a threshold of about 400 molecules of CTBS for water and about 40 molecules for urine. The results are 2–3 orders of magnitude more sensitive than the most sensitive assays currently used. Another assay for botulism toxin yielded similar results.

The technique could be improved by incorporating antibodies into the lipid bilayer and then coding the DNA sequence reporter to match the antibody on the liposome's surface; this would allow simultaneous analysis of multiple antigens. The researchers are currently working on such an approach to detect additional chemical and biological warfare agents, as well as to discover biomarkers for cancer and other diseases.

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